**Iranian Journal of Veterinary Science and Technology**
**Vol. 2, No. 2, 2010, 77-84**

**IJVST**

# The Effect of Storage on the Protein Electrophoretic Pattern in Bovine Serum

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Received: November 15, 2010 Accepted: December 30, 2010

## Abstract

This study was aimed at the evaluation of the influence of storage under various conditions on the relative concentrations of major protein fractions and their proportion in bovine serum. Blood samples were taken from six dairy cattle of a low-land black spotted breed and its crossbreeds. The separated blood serum was fractioned into aliquots. One aliquot was analysed immediately after the separation without storage. The second aliquot was stored at 4 °C for 1 day, the remaining aliquots were kept frozen at -18 °C for 2, 7, and 21 days, and then analysed. Blood serum was analysed by agarose gel electrophoresis for the major protein fractions – albumin (%), alpha-globulins (%), beta-globulins (%), and gammaglobulins (%). Over time, the relative concentrations of albumin in bovine serum showed a tendency of significant decrease during the storage at -18 °C ($p<0.001$). An opposite trend was observed in the percentages of alpha-globulins and gammaglobulins with significant increase of values during the study period ($p<0.05$). In the relative concentrations of beta-globulins in the frozen serum samples, no significant variations were observed. The evaluation of the differences in serum protein fractions between samples without storage and samples stored at 4 °C showed a non-significantly decreased relative concentration of albumin and a significantly increased percentage of gammaglobulin fraction in the refrigerated samples ($p<0.05$). The presented results indicate that the temperature at which serum samples are stored, and the duration of the storage may affect the electrophoretic pattern of serum proteins.

**Keywords:** storage, electrophoresis, protein fraction, dairy cattle

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## Introduction

Measurement of biochemical markers is an important aid to clinicians in the early detection, diagnosis, monitoring, and prognosis of the disease. Whether data are to be used to detect real pathological changes, it is essential that they are appropriate, reliable, and interpreted correctly (Sharma, 2009). The achievement of this goal requires careful attention to every step in the process from the collection of the specimens, their transport to the laboratory, sample handling, and analysis (Marshall and Bangert, 2008). In some cases when the requested test are not available, or the experimental design for retrospective studies necessitates analyses of samples together at later date, there is a need to use stored samples. A lot of authors stated that inadequate biological sample storage, as a potential source of preanalytical errors, may markedly affect the concentrations of many biochemical variables (Jakubowski *et al.,* 1998; Médaille *et al.,* 2006; Ehsani *et al.,* 2008; Cray *et al.,* 2009). However, the influence of sample storage on the main serum protein fractions and their concentrations in veterinary medicine is less well documented.

Determination of the serum protein electrophoretic profile is an important diagnostic aid in clinical biochemistry (Chaudhary *et al.,* 2003). The method can provide valuable information about changes in the concentrations of albumin and alpha-, beta- and gammaglobulins, and thereby help characterize dysproteinemias and protein pattern abnormalities (Riond *et al.,* 2009). Serum electrophoresis is a common technique in the laboratory diagnosis of diseases in human and small-animal medicine (Trumel *et al.,* 1996). However, although it provides useful information about the protein fractions, serum electrophoresis is not commonly used in bovine practice. One of the most important studies that can be performed during the course of the introduction of a new method of the determination of proteins is a stability and storage study.

For this reason, the aim of the present study was to evaluate the influence of storage under various conditions on the relative concentrations of major protein fractions in bovine serum, and thus deepen our knowledge about the stability of the aforementioned important parameters required in the laboratory diagnosis of diseases.

## Materials and methods

Blood samples for the investigations were taken from six dairy cattle of a low-land black spotted breed and its crossbreeds. The evaluated animals were housed on the Clinic for Ruminants of the University of Veterinary Medicine and Pharmacy in Kosice (Slovak Republic), fed twice a day with free access to water. Blood samples were collected by direct puncture of *v.jugularis* into serum gel separator tubes without anticoagulant. In the laboratory, the specimens were immediately centrifuged and the blood serum was fractioned into aliquots. One aliquot was analysed immediately after the separation without storage, and these results obtained at time 0 were considered as initial concentrations. The second aliquot of the separated serum was stored at 4 °C for 1 day, and then analysed. The remaining aliquots were kept frozen at -18 °C, and the concentrations of evaluated parameters were determined after 2, 7, and 21 days.

Blood serum was used for the determination of the relative concentrations (%) of major protein fractions of bovine serum – albumin, alpha-globulins (α-globulins), beta-globulins (β-globulins), and gammaglobulins (γ-globulins). The aforementioned protein fractions of the blood serum were separated by zone electrophoresis on a buffered agarose gel at pH 8.8. The electrophoresis was performed on automated agarose gel electrophoresis system Hydrasys (Sebia Corporate, Evry-Paris, France) using commercial diagnostic kits Hydragel 7 Proteine (Sebia Corporate, Evry-Paris, France) according to the procedure described by the manufacturer. Ten microliters

of each serum sample were applied to performed, numbered sample wells on the agarose gel. Control serum (Control Serum Human Normal, Sebia Corporate, Evry-Paris, France) was included in each run of samples. The electrophoretic migration was performed 15 minutes at 20 °C constantly at 10 W, 40 mA, and 240 V. After migration, the gels were stained in amidoblack staining solution, and then destained with acidic solutions and dried completely. The electroforetic gels were scanned, and the serum protein fractions were visualized and displayed on densitometry system Epson Perfection V700 (Epson America Inc., Long Beach, California, USA) by light transmission and automatic convertion into an optical density curve presentation. Protein fractions were identified and quantified by computer software Phoresis version 5.50 (Sebia Corporate, Evry-Paris, France), and if necessary, corrected by visual inspection of the electrophoretogram. The relative concentrations of the protein fractions were determined as the percentage of the optical absorbance.

Arithmetic means and standard deviations expressed as a percentage for each serum protein fraction and each analysis were calculated using descriptive statistical procedures. The effect of time during the storage of the samples frozed at -18 °C was evaluated by Friedman`s rank sum test. The comparison between the initial relative concentrations and the percentages determined on day 2, 7, and 21 of storage at -18 °C was performed using Wilcoxon matched pairs test. The same test was used for the evaluation of the differences between the initial relative concentrations of protein fractions and the concentrations quantified in serum stored 1 day at 4 °C. All statistical analyses were performed using the programe GraphPad Prism V5.02 (GraphPad Software Inc., California, USA).

## Results

The data referring to the relative concentrations of major protein fractions in bovine serum during storage expressed as average values, standard deviations, including the significance of differences between measured values are presented in Tables 1 and 2.

**Table 1: The changes in the relative concentrations of main serum protein fractions (%) with time during freezer storage (x ± SD)**

| Fraction | Time of analysis (days) | | | | p |
|---|---|---|---|---|---|
| | 0 | 2 | 7 | 21 | |
| albumin | 49.0 ± 10.9 | 47.9 ± 10.3 | 48.1 ± 10.3 | 47.0 ± 10.3* | <0.001 |
| α-globulins | 17.4 ± 2.6 | 18.1 ± 2.6* | 17.9 ± 2.6 | 18.2 ± 2.6* | <0.01 |
| β-globulins | 15.0 ± 2.8 | 15.3 ± 2.2 | 15.2 ± 2.6 | 15.4 ± 2.4 | n. s. |
| γ-globulins | 18.7 ± 7.6 | 18.7 ± 7.4 | 18.8 ± 7.2 | 19.5 ± 7.3 | <0.05 |

p – significance of Friedman´s test
* statistically significant difference compared with the initial relative concentration ($p<0.05$)

**Table 2: Comparison of the relative concentrations of major protein fractions (%) analysed in serum without storage and in serum maintained at 4 °C for 1 day (x ± SD)**

| Fraction | Analysis | | p |
|---|---|---|---|
| | without storage | stored at 4 – 8 °C for 1 day | |
| albumin | 49.0 ± 10.9 | 47.0 ± 10.3 | n. s. |
| α-globulins | 17.4 ± 2.6 | 17.9 ± 2.1 | n. s. |
| β-globulins | 15.0 ± 2.8 | 15.4 ± 2.3 | n. s. |
| γ-globulins | 18.7 ± 7.6 | 19.8 ± 7.7 | <0.05 |

p – significance of Wilcoxon test

The evaluation of the relative concentrations of albumin in bovine serum over time showed a tendency of significant decrease of values during storage at -18 °C ($p<0.001$). The samples maintained in a freezer had reduced concentrations from day 2 onward, with the significantly lowest concentrations on day 21 of storage compared with the initial values ($p< 0.05$). An opposite trend was observed in the relative concentrations of α-globulins during the study period. The percentage of α-globulins in bovine serum increased significantly during the storage at -18 °C ($p<0.01$). For the relative concentrations determined on day 2, a significant increase of these percentages was found ($p<0.05$), and then the values remained stable for the evaluated period of freezer-storage. In the relative concentrations of β-globulins in serum samples stored at -18 °C, no significant variations were observed during the time under study. By the evaluation of the relative concentrations of γ-globulins during the storage at freezer temperature, we observed a stability of measured values for up to day 7 of analysis, showing a more marked increase of percentages on day 21 of storage. The changes in the relative concentrations of γ-globulins in relation to the time of storage at the temperature of -18 °C were significant ($p<0.05$).

The mean relative concentration of albumin obtained in bovine serum stored at 4 °C for 1 day was lower than the average percentage recorded by the evaluation of samples analyzed immediately. When analyzing the relative concentrations of globulin fractions after the storage in refrigerator, no significant differences between the concentrations determined in serum without storage and in samples stored 1 day at 4 °C were found for α- and β-globulin fractions. The evaluation of the concentrations of γ-globulins showed their significantly increased percentages in refrigerated samples ($p<0.05$).

## Discussion

The stability of routine clinical biochemistry parameters (total proteins, albumin, lactate dehydrogenase, creatine kinase, trace elements, hormones) was tested in human and in a range of animal species under different laboratory storage conditions (Jakubowski *et al.,* 1998; Boyanton and Blick, 2002; Cray *et al.,* 2009). These studies stated that the temperature and the duration of storage are important factors which may impact the results of biochemical analyses. Data from such reports regarding the stability of serum protein fractions determined by electrophoresis, and the influence of storage on their concentrations in veterinary medicine are rather scarce. Because serum protein electrophoresis may become of importance in routine laboratory testing also in cattle, it is important to collect information about its biological variation and about the effect of storage on the relative concentrations of protein fractions, as one of the pre-analytical factors that may influence the result of the assay. In the presented study, we observed a slight effect of sample storage at freezer, as well as refrigerator temperatures on the concentrations of some serum protein fractions, characterised by different intensities and redistribution of several electrophoretic bands. In frozen samples, the obtained results showed a trend of decreasing albumin concentrations and increasing percentages of some globulin fractions over time, predominantly α- and γ-globulins. Luraschi *et al.* (2003) reported no significant differences between albumin concentrations determined by electrophoresis during long-term storage at freezer temperatures. Similar findings concerning the storage stability of albumin fraction at refrigerator temperatures were presented by Bossuyt *et al.* (1998). Diminished relative concentrations of albumin at lower temperatures, obtained in our study during refrigerator and freezer storage, may be related to the lability and degradation of this protein resulting from the changes in its molecular

configuration (Schram and Pearson, 2005).

The influence of storage on the distribution of globulin fractions in veterinary medicine is less well documented, and the data are rather contradictory. In the presented study, we observed a trend of increasing relative concentrations of globulin fractions during refrigerator and freezer storage, predominantly in α- and γ-globulins, whereas β-globulins remained relatively stable. This drift in the proportions and redistribution of serum protein pattern after storage of samples may be caused by degradation of albumin, and thus alterations in albumin/globulin ratio. Jenkins and Guerin (1995) and Luraschi *et al.* (2003) observed a progressive decrease of beta-2 fraction in samples maintained at refrigerator temperatures, due to partial degradation of C3 complement, which is the most labile component of this fraction. According to the aforementioned authors, with degradation, the beta-2 fraction may appear distorted with small fractions visualized in the gamma-zone or in beta-1 fraction. Similar findings regarding the degradation of β-region were stated by Jonsson and Carlson (2002) in serum samples maintained at 8 °C on the 1st day of storage. On the other hand, Bossuyt *et al.* (1998) reported that keeping samples for storage at room or refrigerator temperatures may result in a decrease of $\alpha_2$-globulin fraction. The serum protein fractions separated by electrophoresis are quantified to get information for diagnostic or monitoring purposes. This demands precise zone measurement and analytical stability. In our study, the presented results showed that the temperature at which serum samples are stored, and the duration of the storage may affect the electrophoretic pattern of serum proteins with disproportions in the protein fractions. According to Wijnen and van Dieijen-Visser (1996), fresh serum samples are recommended for electrophoretic analyses, because the protein degradation during storage under different conditions may cause alterations in the serum protein profiles. The aforementioned contradictory data indicate that further investigations are needed to clarify the possible influence of storage on the electrophoretic pattern of serum proteins, and to explain the changes in the proportion of protein fractions during storage.

In conclusion, our results indicate that proper sample handling, and the time and duration of storage are important factors also for electrophoretic analyses. In our study, the presented results showed some effects of sample storage at freezer, as well as refrigerator temperatures on the changes in the relative concentrations of some protein fractions in bovine serum. Thus, sample processing can affect the electrophoretic pattern of serum proteins, and the ability to differentiate between sick and healthy animals. Therefore, these data suggest that the storage temperature and time should be taken into consideration, when interpreting the results from electrophoretic analyses, when the analysis is not available immediately after sample collection.

## Acknowledgments

This work was supported by VEGA Scientific Grant No 1/0614/09 from the Ministry of Education.

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**Iranian Journal of Veterinary Science and Technology**
**Vol. 2, No. 2, 2010, 77-84**

**IJVST**

# تاثیر شرایط مختلف نگهداری بر روی الگوی الکترفورتیک پروتئین در سرم گاو

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*دریافت مقاله: ۸۹/۸/۲۴ پذیرش نهایی: ۸۹/۱۰/۹*

## چکیده

مطالعه حاضر به منظور بررسی اثرات شرایط مختلف نگهداری بر روی غلظت های نسبی و اجزای اصلی پروتئینی سرم گاو انجام گرفت. متعاقب خونگیری از شش راس گاو شیری نژاد low-land black spotted breed، سرم جداسازی شده و به چند بخش تقسیم شد. یکی از نمونه ها بلافاصله بدون ورود به مرحله ذخیره سازی مورد آنالیز قرار گرفت. نمونه دوم به مدت یک روز در دمای ۴°C نگهداری شد، در حالیکه سایر نمونه ها در دمای ۱۸°C- برای ۲، ۷ و ۲۱ روز نگهداری شده، سپس فراکسیون های آلبومین (٪)، آلفاگلوبولین ها (٪)، بتاگلوبولین ها (٪) و گاماگلوبولین ها (٪) با استفاده از الکتروفورز ژل آگاروز مورد آنالیز قرار گرفتند. با گذشت زمان، کاهش معنی داری در غلظت های نسبی آلبومین سرم گاو متعاقب نگهداری در دمای ۱۸°C- رخ داد ($p<0.001$). در حالیکه غلظت های آلفاگلوبولین ها و گاماگلوبولین ها روند متفاوتی را نشان داده و در طول مدت مطالعه افزایش یافت ($p<0.05$). هیچ تغییر معنی داری در غلظت های نسبی بتاگلوبولین ها مشاهده نشد. بررسی تغییرات اجزای پروتئینی سرم بین نمونه های تازه و نمونه های نگهداری شده در دمای ۴°C حاکی از کاهش غیر معنی دار غلظت های نسبی آلبومین و افزایش درصد فراکسیون گاماگلوبولین در نمونه های نگهداری شده در یخچال بود ($p<0.05$). نتایج مطالعه حاضر نشان داد که دما و مدت زمان نگهداری نمونه های سرم می توانند بر روی تابلوی الکتروفورزی پروتئین های سرم تاثیر گذار باشند.

**واژگان کلیدی:** نگهداری، الکتروفورز، فراکسیون پروتئینی، گاو شیری